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White salted noodle characteristics from transgenic isolines of wheat over expressing puroindolines

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ABSTRACT

The closely linked genes puroindoline a (*Pina*) and puroindoline b (*Pinb*) control most of the variation in wheat (*Triticum aestivum)* grain texture. Mutations in either *Pina* or *Pinb* result in hard grain with wild type forms of both genes giving soft grain. Asian noodles are prepared from both hard and soft classes of wheat. Our objective was to examine color and texture characteristics of white salted noodles processed from flours of transgenic isolines of Hi-Line hard red spring wheat over expressing *Pina-D1a*, *Pinb-D1a* or both and a control giving a range in grain texture from very soft to hard. White salted noodles were prepared and color and texture characteristics were measured. The three softer textured transgenic isolines showed greater change in L^* with time than Hi-Line. The noodles were more adhesive (more negative value), firmer, and chewier as the grain texture became successively softer when cooked at 5 min. These texture differences were not as apparent when noodles were cooked for an optimum time. Starch pasting properties did not explain the noodle textural differences. A possible explanation for the noodle texture differences may be related to starch damage which ranged from 2.2% for HGAB to 6.7% for Hi-Line, flour particle size differences and subsequent water absorption differences among the four genotypes. Over expression of puroindolines did not enhance quality of white salted noodles when prepared under these conditions.

1. Introduction

The texture of wheat (*Triticum aestivum*) endosperm is related to the strength of the bond between starch granules and the surrounding protein matrix (Barlow et al., 1973). In hard wheats the endosperm is a continuous matrix of starch and protein, while in soft wheats starch granules and the protein matrix appear discontinuous. In soft wheats starch granules are easily dislodged intact and undamaged from the protein matrix during milling. On the other hand, the starch granules are tightly bound to the protein matrix in hard wheats leaving them more susceptible to fracture during milling. Because flours from the two textural classes have different physical properties, soft wheats are best suited for cookies, cakes and pastries, while hard wheats are used for leavened products such as bread and bread products (reviewed in Morris and Rose, 1996; Pomeranz and Williams, 1990).

Asian noodles are unique among wheat flour based products in that they are made from both soft and hard wheat flours. The basic ingredients are water, salt and flour. The type of salt, properties of the flour and manufacturing process lead to a wide array of noodle types (Hou and Kruk, 1998). Quality of noodles is based on appearance, primarily color, and texture. High protein hard wheats produce noodles with a more firm texture than those from lower protein soft wheats. Consumers universally prefer noodles that are bright and maintain their color with time. Desired textural characteristics vary by noodle type and geographic region. Differences in starch damage, particle size, and associated water hydration traits may be partially responsible for noodle textural differences, although differences in protein content between hard and soft wheat classes contribute to textural differences.

The Hardness (*Ha*) locus on chromosome 5D is responsible for most of the variation in grain texture in wheat (Law et al., 1978; Mattern et al., 1973). Greenwell and Schofield (1986) identified friabilin as a marker protein for grain softness which was present in large amounts on the surface of water-washed starch of soft wheats and nearly absent from hard wheats (Bettge et al., 1995; Greenblatt

Abbreviations: FSV, flour swelling volume; L-DOPA, 3,4-dihydroxy-L-phenylalanine; MOPS, 3-[*N*-morpholino] propane sulfonic acid; PINA, puroindoline a; PINB, puroindoline b; PPO, polyphenol oxidase; RVA, Rapid Visco Analyzer; SKCS, single kernel characterization system.

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et al., 1995; Morris et al., 1994). Friabilin is composed of two major polypeptides termed puroindoline a (PINA) and puroindoline b (PINB). Genes coding for these two proteins, *Pina* and *Pinb*, are tightly linked to the Ha locus on chromosome 5D (Giroux and Morris, 1997; Sourdille et al., 1996) and probably functions together as the *Ha* locus (Giroux and Morris, 1998). Recent results have shown that mutations in either *Pina* or *Pinb* are associated with the hard phenotype (Giroux and Morris, 1997, 1998; Lillemo and Morris, 2000; Morris et al., 2001). The glycine-to-serine mutation in *Pinb* (*Pinb-D1b* allele) and the null mutation for *Pina* (*Pina-D1b* allele) are the two most common mutations among US hard wheats (Morris et al., 2001). Puroindolines are believed to mediate the starch protein interaction by binding with polar lipids at the surface of starch granules (Gautier et al., 1994; Greenblatt et al., 1995; Marion et al., 1994). These biochemical differences provide the basis for differences in starch damage, water hydration traits, and end product uses of hard and soft wheat.

In wheat, transgenic expression of wild type *Pinb-D1a* sequence in the hard spring wheat 'Hi-Line' complemented the glycine-to-serine mutation (*Pinb-D1b* allele) resulting in a soft phenotype (Beecher et al., 2002). Hogg et al. (2004) expressed *Pina-D1a*, *Pinb-D1a*, or both in the same Hi-Line background. Expression of either *Pinb*, or both *Pina* and *Pinb* gave a soft phenotype, while *Pina* alone was intermediate in grain texture. Transgenic expression of wild type *Pina-D1a* sequence in the hard wheat 'Bobwhite' which has the *Pina-D1b* (null) allele also gave a soft phenotype (Martin et al., 2006). Swan et al. (2006b) crossed Hi-Line transgenic lines expressing *Pina* or *Pinb* to a soft wheat and found progeny with added *Pinb* had softer grain than those with added *Pina*. They concluded *Pinb* may be more limiting than *Pina* to grain softness in soft wheats. Wanjugi et al. (2007) also crossed Hi-Line transgenic isolines with either added *Pina* or *Pinb* to *Pina* null and *Pinb* null genotypes. Soft grain was obtained in progeny where both *Pin* genes were functional, and intermediate grain texture was obtained when only one *Pin* was over expressed in the presence of the same native *Pin*. These experiments have shown that the soft phenotype can be restored by complementing mutated *Pinb* and null *Pinb* or a null *Pina* allele with the corresponding wild type *Pin* allele. Quantification of total and starch bound PINA and PINB (friabilin) has shown that grain texture is not related to total PIN. But rather the soft phenotype occurs when both proteins bind to starch forming friabilin.

Previous work has demonstrated that changing grain hardness by over expressing puroindolines in transgenic isolines (Hogg et al., 2005; Martin et al., 2007) and allelic variation at *Pin* loci in a recombinant inbred population (Martin et al., 2001) affected bread quality. Swan et al. (2006a) showed that over expression of puroindolines also slowed dry matter digestibility and starch digestibility in the rumen. Several studies have investigated Asian noodle properties using flour from hard and soft wheat. Few studies have isolated the effect of grain texture on noodle quality using populations with a defined genetic structure. Evaluation of flour quality and flour-water slurry from a hard (*Pina-D1a*/*Pinb-D1b*) by soft (*Pina-D1a*/*Pinb-D1a*) wheat cross showed that wild type *Pina-D1a*/*Pinb-D1a* gave lower flour yield and flour protein, decreased ash and gave flour that was darker (lower L^*) and more yellow ($>b^*$) than the hard *Pina-D1a*/*Pinb-D1b* group (Nagamine et al., 2003). Storlie et al. (2006) did sensory evaluation of white salted noodles made from flours of hard and soft bulk segregants from a hard (*Pina-D1a*/*Pinb-D1b*) by soft (*Pina-D1a*/*Pinb-D1a*) cross. The evaluators perceived noodles from the soft (*Pina-D1a*/*Pinb-D1a*) to be softer than noodles from the hard (*Pina-D1a*/*Pinb-D1b*) bulks. The observed puroindoline effect on noodle texture may have been confounded by flour protein differences brought about by differences in flour yield between the two classes.

Since puroindoline over expression has been shown to affect bread quality and ruminal digestibility by beef cattle, it seems that Asian noodle quality may be affected as well. Our objective was to examine color and texture characteristics of white salted noodles processed from flours of transgenic isolines of wheat over expressing *Pina-D1a*, *Pinb-D1a* or both and a control giving a range in grain texture from very soft to hard.

2. Experimental

Hi-Line hard red spring wheat (PI 549275) (Lanning et al., 1992) and three transgenic isolines (HGA3, HGB12, and HGAB18) were grown in two replications of a randomized complete block design in both rain-fed and irrigated environments at the Arthur H. Post Field Research Farm near Bozeman, MT in 2004. HGA3 over expresses *Pina-D1a*, HGB12 over expresses *Pinb-D1a*, and HGAB18 over expresses both *Pina-D1a* and *Pinb-D1a*. The derivation and characterization of these transgenic isolines are described in Hogg et al. (2004, 2005). Grain texture and kernel weight were determined using the Perten Single Kernel Characterization System (SKCS) 4100 (Perten Instruments, Springfield, IL) on 100 seeds per replication. Grain protein content was determined on grain from each replication by near-infrared spectroscopy for whole grain using the Tecator Infratec 1225 Grain Analyzer (Foss North America, Silver Spring, MD).

Approximately 31 kg of cleaned grain from each replication was tempered to 13.5% for HGB12 and HGAB18 and 15.5% moisture for HGA3 and Hi-Line by adding water. Each replication was milled on a Miag Multomat pilot scale mill (Posner and Hibbs, 1997). The mill produces 10 flour streams and four feed streams from three break and five reduction rolls. Feed rate was 920–980 g min^{-1}. Break rolls were adjusted so that material flow through the mill was balanced while still achieving good bran clean-up characteristics without excessive shattering. The target first break release was for 43% of the grind to pass through a #24 Tyler (707 μm) wire screen in 20 s of sifting. The target second break release was for 64% of the grind to pass through the above sifting. The third break roll was adjusted to clean the bran as completely as possible without excessive shattering. The adjustment for reduction rolls was done by observation of stock with the objective of making as much flour as possible by the end of the process, but not to the point of overgrinding and flaking the stock.

The straight grade flour used was a composite of the 10 flour streams blended together using a horizontal ribbon blender. Ash content was measured on 3–5 g sample ignited and heated for 18 h at 580 °C in a muffle furnace (AACC Method 08-01). Flour protein content was determined using 0.25 g flour samples and a LECO FP-528 nitrogen analyzer (LECO Corp., St. Joseph, MI). Protein content was obtained as nitrogen in g $kg^{-1} \times 5.70$ with flour protein corrected to a 14.0% moisture basis (AACC Method 46-30). These measurements were obtained from each replication.

White salted noodles were prepared using 100 g straight grade flour (14% moisture basis, fwb) and 29.2 ml salt (NaCl) water solution (4.29% w/v) added to the flour during a 30 s time period. Doughs were mixed in a Finney Special mixer (100 g Micro Dough pin mixer, head speed 102 rev/min (National Manufacturing Co., TMCO, Inc., Lincoln, NE)) for 5 min and 45 s. Flour adhering to the inside of the mixing bowl and pins was brushed down and premixed for 15 s prior to adding the salt–water solution. The salt–water solution was slowly added so that the last drop was added 30 s after the initial drop was dispensed to insure uniform hydration and complete incorporation. After 30 additional seconds of mixing, the mixer was stopped to clean dough off the pins and to break up any large lumps of dough. This step was repeated after an additional 1 min and 30 s of mixing followed by a final 3 min of mixing. The crumbly dough was pressed by hand into a cohesive

rectangular block then placed in a plastic bag to rest for 30 min at room temperature. The dough block was then passed though a laboratory noodle machine (Otake Noodle Machine Manufacturing Co., Ltd., Tokyo, Japan) with an initial gap of 3 mm. The dough was then book-folded once then passed through a 5 mm gap three times and placed in a plastic bag for 30 min at room temperature. The sheet was progressively reduced by sheeting the dough through the following gaps; 4 mm, 3 mm, 2 mm, and 1.5 mm. Two pieces of sheeted dough were reserved for 0 h and 24 h color measurements. The final sheeting thickness was 1.2 mm which was checked in five places using a Pocket Dial Gauge 1010MZ (L.S. Starrett Company Athol, MA). The rectangular dough sheet was then cut into 2.5 mm wide noodles and stored in a plastic bag for 24 h before cooking and texture evaluation. Dough sheet color measurements were taken on each side of the two reserved dough sheets at 0 h and 24 h after sheeting with a Minolta CR-310 Chroma Meter (Minolta, Ramsey, NJ). The Minolta Chroma Meter uses the Commission Internationale de l'Eclairage (CIE) color system and was used to measure L^* (brightness) a^* (red–green) b^* (yellow–blue). More positive values of L^*, a^* and b^* indicate increasing white, red and yellow, respectively. Noodles were cooked by adding 50 g of noodles to 500 ml of boiling distilled water for 5 min. After 5 min of boiling, noodles were removed from the heat. Noodles were transferred to a thermal cup and remained in a portion of the water they were boiled in for 5 min. Noodles were transferred to a basket strainer then rinsed in an ice water bath with 10 s of agitation using chopsticks. The strainer was removed from the ice water and tapped 10 times to remove excess water from the noodles before texture analysis. Texture profile analysis (springiness, cohesiveness, hardness and chewiness) was performed immediately on five strands of rinsed noodles using a TA-XT2 Texture Analyzer and Texture Exponent software (Texture Technologies Corp., Scarsdale, NY) then averaged to obtain texture readings. The method for obtaining the texture measurements was the same as that described by Epstein et al. (2002). Noodles were prepared and color and texture measurements obtained from straight grade flour two times from each replication. The two times were considered repeated measures.

The same straight grade flour source for each replication was used to determine texture characteristics when noodles were cooked to an optimum cooking time. The noodles were prepared exactly as described above, except that each noodle sample was cooked to an optimum cooking time. Optimum cooking was determined as when noodles were completely translucent throughout when cut with a laboratory watch glass. The texture measurements were obtained as described above.

Flour swelling volume (FSV) was obtained for both flour and primary prime starch. Primary prime starch was obtained from wet milling of flour using a dough-dispersion and centrifugation method (Sayaslan, 2006), an adapted method from Czuchajowska and Pomeranz (1993), with some minor modifications. Flour (50–75 g, 14% mb) was mixed in an ML-33777 N50 Hobart mixer (Troy, Ohio) with gradual addition of water (45–50 ml, 25 °C) until the mixture became a cohesive, stiff dough, and cleaned itself from the mixing bowl (3–4 min). The developed stiff dough was covered with 150 ml water at 25 °C and was rested at room temperature for 30 min. The dough and liquid were transferred to a blender (TSK-9368AP, China) and dispersed at high speed for 1 min. The slurry was transferred to 250 ml Sorvall centrifuge bottles and centrifuged at 2500 × g at 25 °C for 15 min using a Sorvall super T21 centrifuge (Kendro Laboratory Products, Newtown, CT), then the supernatant was weighed and discarded. The top layer, which consisted mainly of gluten, insoluble pentosans, damaged starch, and small granular starch, was carefully removed from the bottom layer which consisted of primary prime starch. The primary prime starch was weighed and dried for 2 days at 37 °C using a forced air incubator. Flour swelling volume was performed using 0.45 g flour or starch on a dry weight basis well dispersed in 12.5 mL water. The samples were placed in a hot water bath at 92.5 °C, and were continuously inverted for 30 min. A rapid cooling in an ice water bath followed by 5 min in 25 °C water was used to bring the samples to room temperature. Then the samples were centrifuged at 1000 × g for 15 min. The height of the gel was read in mm and reported in mL/g using the following conversion formula: ((mm * 1.52) − 0.30 mL)/0.45 g.

Hot-pasting viscosity was measured by the Rapid Visco Analyzer (RVA), using 3.5 g of flour on a 14% moisture basis, in 25 mL water. The peak viscosity in RVA units (centipoise × 10) was recorded. The temperature profile used to obtain the peak viscosity consisted of 2 min at 60 °C, then constant rate heating to 93 °C in 6 min with a final hold of 2 min at 93 °C, for a total of 10 min.

Polyphenol oxidase (PPO) in straight grade flour was measured for each replication using 100 mg flour added to 2 ml deep wells in a 96 well plate. Flour samples were stained by adding 0.5 ml of solution with 5 mM L-DOPA (3,4-dihydroxy-L-phenylalanine) substrate in 50 mM MOPS (3-[*N*-morpholino] propane sulfonic acid) buffer at pH 6.5 to each well. The plate was shaken for 7 min on a Beadbeater and then left to sit on counter for 10 min. Samples were centrifuged at 3500 × g for 5 min. Solutions (200 μl) were transferred to a clean 96 well plate. Change in absorbance was compared to a substrate only control. Change in absorbance was recorded at 475 mm using a SPECTRAmax PLUS^{384} spectrophotometer (Molecular Devices, Sunnyvale, CA).

Grain texture, seed weight, grain protein flour color variables, RVA variables, PPO activity, and swelling volumes were analyzed via analysis of variance for a randomized complete block design combined over environments using PROC MIXED in SAS (SAS Institute, 2004). All variables for noodle color and texture were analyzed where the model was analogous to a randomized block split plot combined over environments where genotypes were main plots and two times were subplots using PROC MIXED in SAS (SAS Institute, 2004). All factors except replication were considered fixed effects. Comparisons between genotype means were made using least significant difference (LSD).

3. Results

Hi-Line had the hardest grain, HGA3 was intermediate, and HGB12 and HGA3B18 had soft grain (Table 1). All genotypes were different from each other in grain texture except for the difference between HGB12 and HGAB18 ($P = 0.052$). These results agree with earlier reports for these same genotypes where addition of *Pinb* or both *Pina* and *Pinb* produced the softest grain and addition of *Pina* produced intermediate grain texture (Hogg et al., 2004, 2005). For seed weight and grain protein, HGA3 had lower seed weight, and HGAB18 had higher grain protein content than Hi-Line. Otherwise transgenic lines were similar to Hi-Line. The rain-fed environment had lower kernel weight but higher grain protein than the irrigated environment, but genotypes did not interact with environment for these kernel traits.

Significant differences ($P < 0.01$) among the four genotypes were detected for flour protein, ash and color except for a^* ($P = 0.06$) and flour PPO (Table 2). HGB12 had lower flour protein than the other three flours. Hi-Line and HGB12 straight grade flours were nearly identical in ash content, HGA3 was intermediate and HGAB18 was the lowest in ash content. The flour protein and ash values differ slightly from those reported in Martin et al. (2007), because they were determined directly from straight grade flour rather than indirectly from cumulative values from flour mill streams as in Martin et al. (2007). Hi-Line flour was darker (lower L^*) than HGA3 which in turn was darker than HGB12 and HBAB18. On the other hand, Hi-Line flour had greater b^* (more yellow) with

Table 1
Means for kernel characteristics for Hi-Line hard red spring wheat and transgenic isolines over expressing puroindoline a (HGA3), puroindoline b (HGB12), or both puroindoline a and b (HGAB18) averaged over two replications for rain-fed and irrigated environments at Bozeman, MT

Mean	Grain hardness[a]	Kernel weight (mg)	Wheat protein (%)
Genotype			
Hi-Line	73.7	35.0	14.4
HGA3	42.0	33.6	14.3
HGB12	9.7	35.4	14.4
HGAB18	6.4	34.3	14.7
Genotype *P* value	<0.01	0.01	0.01
LSD(0.05)	3.3	0.8	2
Environment			
Rain-fed	34.2	31.0	15.2
Irrigated	31.7	38.1	13.7
Environment *P* value	0.29	0.01	<0.01
$G \times E$ *P* value	0.09	0.12	0.06
CV%	5.8	1.4	0.7

[a] Measured by single kernel characterization system.

Table 3
Means for white salted noodle color characteristics for Hi-Line hard red spring wheat and transgenic isolines over expressing puroindoline a (HGA3), puroindoline b (HGB12), or both puroindoline a and b (HGAB18) averaged over two replications for rain-fed and irrigated environments at Bozeman, MT

Mean	L^*, 0 h	L^*, 24 h	L, 0 −24 h	a^*, 0 h	a^*, 24 h	a, 0 −24 h	b^*, 0 h	b^*, 24 h	b^*, 0 −24 h
Genotype									
Hi-Line	83.8	72.5	11.3	1.44	2.69	−1.25	13.9	18.7	−4.8
HGA3	83.4	70.0	13.4	1.63	3.59	−1.96	13.4	18.3	−4.9
HGB12	83.7	69.5	14.2	1.62	3.61	−1.99	12.5	16.7	−4.2
HGAB18	84.3	70.3	14.0	1.43	3.39	−1.96	12.5	17.0	−4.5
Genotype *P* value	0.06	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	0.08
LSD(0.05)	0.7	0.79	0.3	0.07	0.11	0.06	0.6	0.2	0.60
Environment									
Rain-fed	83.4	69.6	13.5	1.63	3.51	−1.88	13.5	18.1	−4.6
Irrigated	84.5	71.5	12.9	1.43	3.13	−1.70	12.6	17.2	−4.6
Environment *P* value	0.01	0.02	0.06	0.02	0.04	0.06	0.01	<0.01	0.91
$G \times E$ *P* value	0.54	0.73	0.67	0.58	0.05	<0.01	0.78	0.20	0.64
CV%	0.3	0.8	2.7	4.0	2.2	2.6	3.4	0.66	9.3

HGA3 intermediate and HGB12 and HGAB18 the lowest b^*. The rain-fed environment had higher grain protein than the irrigated environment. Genotypes did not interact with environment for any of the flour related traits.

The initial noodle L^* measurements showed that genotypes differed relatively less ($P = 0.06$) than at 24 h ($P < 0.01$) (Table 3). After 24 h noodles from all three transgenic lines were darker than Hi-Line. Thus, all three transgenic lines showed more change in L^* than Hi-Line. Among the three transgenic lines HGA3 had less change in L^* than HGAB18 and HGB12. Noodles acquired more red color ($>a^*$) with time, and genotypes were different ($P < 0.01$) for a^* at 0 h, 24 h and change with time (0–24 h). Hi-Line showed less change in a^* with time than did the three transgenic isolines. The genotypes reacted differentially between environments only for change in a^* with time. The means were −1.25, −2.04, −2.14, and 2.08 for rain-fed, −1.26, −1.82, −1.83, and −1.83 for irrigated for Hi-Line, HGA3, HGB12, and HGAB18, respectively. Hi-Line showed less change in a^* than the three transgenic lines ($P < 0.01$) in both environments. The reason for the interaction is that Hi-Line had essentially the same change while the three transgenic lines had greater change in rain-fed than the irrigated environment. Noodles also acquired more yellow ($>b^*$) with time. Hi-Line and to a lesser extent HGA3, had the highest b^* at both 0 h and 24 h than HGB12 and HGAB18. Differences among genotypes for change in b^* were less pronounced ($P = 0.085$) than for change in L^* and a^*.

Genotypes did interact with environment for many of the noodle texture measurements when cooked for 5 min (Table 4). Therefore, means for each environment are presented (Table 4). We did not detect differences among the genotypes for springiness in either environment. For cohesiveness, the genotypes differed only for the rain-fed environment. Where the genotypes did differ, Hi-Line (the hardest grain) was less cohesive than HGAB18 (the softest grain). Similar to cohesiveness, genotypes differed only for rain-fed environment for adhesiveness. Hard textured Hi-Line made noodles less adhesive than soft textured HGB12 and HGAB18. Hi-Line made softer noodles than the three transgenic lines for both environments. Differences among the genotypes were detected for chewiness in both environments. Hi-Line noodles were less chewy than HGAB18 noodles. The nature of the genotype by environment interaction was that genotype differences were less pronounced in the irrigated environment than the rain-fed environment. However, overall conclusions were similar when averaged over environments. When cooked for optimum cooking time, the same trends were apparent, but we were not able to detect differences among genotypes (Table 5). The mean optimum cooking time was 6.3 min, and the genotypes did not differ in optimum cooking time. The mean values for the rain-fed and irrigated environments and generally for the four genotypes were similar between the 5 min and optimum cooking times for all the noodle texture traits.

Among the starch pasting properties measured by RVA, differences among genotypes were observed for RVA breakdown and peak time (Table 6). HGA3 had higher breakdown than the other three, while HGAB18 and Hi-Line had lower peak time than the other two genotypes. Flour swelling volume was the least for Hi-Line and the greatest for HGB12 and HGAB18. On the other hand, starch swelling volume did not differ among the genotypes. These traits all reacted similarly across environments.

Table 2
Means for flour characteristics for Hi-Line hard red spring wheat and transgenic isolines over expressing puroindoline a (HGA3), puroindoline b (HGB12), or both puroindoline a and b (HGAB18) averaged over two replications for rain-fed and irrigated environments at Bozeman, MT

Mean	Flour protein (%)	Flour ash (%)	Flour yield[a] (%)	Flour PPO[b] (ΔA_{475} g flour^{-1} min^{-1})	Flour L^*	Flour a^*	Flour b^*
Genotype							
Hi-Line	13.5	0.49	74.4	0.105	90.9	−0.632	7.39
HGA3	13.4	0.47	73.2	0.118	91.6	−0.602	6.02
HGB12	12.9	0.49	71.4	0.110	92.0	−0.575	5.15
HGAB18	13.3	0.45	71.1	0.125	92.1	−0.630	5.21
Genotype *P* value	0.01	<0.01	<0.01	0.54	<0.01	0.06	<0.01
LSD(0.05)	0.31	0.02	1.1	0.035	0.2	0.04	0.13
Environment							
Rain-fed	13.9	0.47	71.9	0.117	91.6	−0.611	6.07
Irrigated	12.6	0.48	73.1	0.112	91.7	−0.609	5.81
Environment *P* value	0.01	0.14	0.16	0.71	0.70	0.88	0.02
$G \times E$ *P* value	0.57	0.08	0.35	0.46	0.26	0.90	0.75
CV%	1.5	2.2	1.0	17.6	0.2	4.1	1.2

[a] From Martin et al. (2007).
[b] Polyphenol oxidase.

4. Discussion

Our goal was to determine the impact changes in grain texture have on white salted noodle color and texture. We accomplished

Table 4
Means for white salted noodle texture characteristics for Hi-Line hard red spring wheat and transgenic isolines over expressing puroindoline a (HGA3), puroindoline b (HGB12), or both puroindoline a and b (HGAB18) averaged over two replications for rain-fed and irrigated environments at Bozeman, MT

Environment	Genotype	Springiness	Cohesiveness	Adhesiveness	Hardness (g)	Chewiness
Rain-fed	Hi-Line	0.916	0.580	−33.8	1154.1	612.6
	HGA3	0.925	0.587	−41.2	1238.3	672.6
	HGB12	0.926	0.599	−57.0	1326.4	736.1
	HGAB18	0.932	0.605	−59.2	1383.2	778.6
	Genotype *P* value	0.79	0.03	0.01	<0.01	<0.01
	LSD(0.05)	0.038	0.015	12.7	53.8	58.1
Irrigated	Hi-Line	0.926	0.593	−41.6	1128.6	619.6
	HGA3	0.916	0.581	−46.9	1221.5	649.7
	HGB12	0.914	0.580	−49	1211.9	642.5
	HGAB18	0.905	0.588	−47.7	1275	678.7
	Genotype *P* value	0.62	0.24	0.54	<0.01	0.2
	LSD(0.05)	0.038	0.015	12.7	53.8	58.1
Combined	Hi-Line	0.921	0.586	−37.7	1141.4	616.1
	HGA3	0.920	0.584	−44.1	1229.9	661.2
	HGB12	0.920	0.589	−53	1269.2	689.3
	HGAB18	0.919	0.596	−53.5	1329.1	728.7
	Genotype *P* value	0.99	0.12	0.01	<0.01	<0.01
	LSD(0.05)	0.027	0.011	9.0	38.1	41.1
	Environment					
	Rain-fed	0.925	0.593	−47.8	1275.5	700
	Irrigated	0.916	0.585	−46.3	1209.3	647.6
	Environment *P* value	0.10	0.15	0.37	0.04	0.06
	$G \times E$ *P* value	0.39	0.04	0.06	0.04	0.05
	CV%	2.1	1.5	15.5	2.3	4.0

this by using transgenic isolines in the hard wheat cultivar Hi-Line over expressing *Pina-D1a*, *Pinb-D1a* or both. These four genotypes gave grain texture from hard to very soft. These lines were tempered according to their grain texture, and milled on a long flow pilot scale mill (Martin et al., 2007). These lines being transgenic isolines should test the effect of puroindoline over expression and associated effects on white salted noodle quality without other confounding genetic factors.

Flour was brighter ($>L^*$) but had less yellow ($<b^*$) as grain texture got softer for the four genotypes (Table 2). Flour brightness is influenced mainly by bran contamination, reflected in ash content, and yellowness is imparted by xanthophylls, namely lutein and its fatty acid esters, in the endosperm (Mares and Campbell, 2001). The differences in flour L^* values may reflect the differing ash content with HGAB18 being the brightest and the lowest in ash and Hi-Line the darkest and the highest in ash. Although particle size distribution was not measured, hard textured Hi-Line would have coarser particle size than soft textured HGB12 and HGAB18. Symons and Dexter (1991) found that L^* increased and b^* decreased as flour particle size became smaller with a constant ash content. Our differences among genotypes in flour L^* and b^* may reflect particle size differences.

Over expression of puroindolines impacted the noodle color profile as well. After 24 h the softer genotypes with added puroindoline produced noodles that were darker, with more red but less yellow color than hard Hi-Line (Table 3). The differences in noodle b^* at 0 h and 24 h reflect the same relative differences as for flour b^*. The progressive increase in difference between 0 h and 24 h for L^* with progressive reduction in grain texture from Hi-Line to HGAB18 was unexpected. Factors affecting time-dependent change in noodle brightness (L^*) include flour protein content and PPO enzyme activity. Habernicht et al. (2002) showed that noodle brightness was highly negatively correlated with increasing flour protein content within a cultivar. Flour protein differences among the four genotypes, though comparatively small, were largely because soft genotypes HGAB18 and HGB12 were lower than the other two genotypes. Yet, these genotypes had the lowest L^* at 24 h and the greatest change in L^* with time. PPO activity has been shown to be positively related to time-dependent discoloration of noodles (Baik et al., 1995; Kruger et al., 1994; Park et al., 1997), and PPO activity tends to increase with flour extraction rate (Hatcher and Kruger, 1993). Flour extraction rate, ranged from 71.1% for HGAB with the softest grain to 74.4% for Hi-Line with the hardest grain. Our data showed no difference in PPO activity among the four genotypes. The differences observed for noodle brightness at 24 h, and changes in noodle brightness with time are in the opposite direction from that expected if flour protein or PPO activity differences were the cause. In a previous study comparing genotypes selected for high versus low PPO activity where noodles were prepared using the same protocol as in the present study, we found change in L^*, a^*, and b^* for high and low selections was 9.6 and 8.9; −0.62 and −0.74; and −7.02 and −7.07, respectively (Martin et al., 2005). Those color changes between 0 h and 24 h were similar to ours where PPO activity was not a factor.

Noodles were cooked for constant 5 min period, and then in a second trial they were cooked for an optimum time. The genotypes were not different in optimum cooking time. This essentially gave two cooking times, 5.0 and 6.3 min. Noodle texture characteristics were modified by over expression of puroindolines when cooked for a constant 5 min. Noodle hardness and chewiness increased, and adhesiveness became more negative progressively from hard (Hi-Line) to very soft (HGAB18) grain. But no significant change in springiness and cohesiveness was detected. Though compressed in magnitude and not statistically significant the same trend for adhesiveness and hardness was apparent for optimum cooking time as for the 5 min cooking time. Data precision, as measured by coefficient of variation, was similar between the two cooking times. With additional replications, these differences may have been detected as they were at the 5 min cooking time.

We did observe differences for RVA breakdown and peak time, but these differences were not related to the grain texture differences or the noodle texture differences at the 5 min cooking time.

Table 5
Means for white salted noodle texture characteristics at optimum cooking time for Hi-Line hard red spring wheat and transgenic isolines over expressing puroindoline a (HGA3), puroindoline b (HGB12), or both puroindoline a and b (HGAB18) averaged over two replications for rain-fed and irrigated environments at Bozeman, MT

Mean	Cooking time (min)	Springiness	Cohesiveness	Adhesiveness	Hardness (g)	Chewiness
Genotype						
Hi-Line	6.12	0.936	0.605	−39.9	1180.7	669.5
HGA3	6.50	0.936	0.602	−44.3	1225.5	691.4
HGB12	6.38	0.945	0.608	−48.5	1265.7	728.1
HGAB18	6.13	0.939	0.615	−52.3	1257.2	727.5
Genotype *P* value	0.79	0.19	0.48	0.07	0.20	0.24
LSD(0.05)	1.10	0.009	0.021	9.6	96.8	73.3
Environment						
Rain-fed	6.50	0.947	0.613	−47.4	1257.0	730.4
Irrigated	6.06	0.932	0.615	−45.2	1207.6	677.8
Environment *P* value	0.19	0.21	0.30	0.70	0.21	0.23
$G \times E$ *P* value	0.55	0.45	0.47	0.3	0.96	0.90
CV%	10.2	0.6	1.9	12.0	4.5	6.0

We also found that flours from the softest wheats, HGB12 and HGAB18 swelled more than flour from hard wheat Hi-Line, yet the four genotypes were essentially equal for starch swelling volume. Previous experiments have indicated that swelling of flour and starch is highly correlated (Wang and Seib, 1996). The disparity between flour and starch swelling may be because protein and/or lipids present on starch granules in flour may have been removed during preparation of water-washed starch. In addition, once starch is isolated potential confounding effects from flour particle size and starch damage differences would be equalized. The amylose to amylopectin ratio is a major factor affecting starch pasting and swelling and ultimately noodle texture differences (Epstein et al., 2002). Amylose content in the wheat endosperm is controlled by allelic variation at the waxy (*Wx*) loci. Amylose is reduced in genotypes with the null mutation at one or more of the three *Wx* loci (*Wx-A1*, *Wx-B1*, and *Wx-D1*) (Nakamura et al., 2002). Flours and starch from "partial waxy" genotypes have reduced amylose, increased swelling, and altered starch pasting properties including higher peak paste viscosity, lower final viscosity, greater breakdown, and lower onset temperature. Although amylose content was not measured, these four genotypes did not vary at the *Wx* loci. It seems unlikely that differences in amylose could explain the observed differences for RVA breakdown, peak time, or texture differences observed for the 5 min cooking time.

Noodles in this trial were prepared with a constant amount of water added to flour. Water absorption can influence both color and texture of white salted noodles (Hatcher et al., 1999; Park and Baik, 2002). Hatcher et al. (1999) found that white salted noodles became darker ($<L^*$) with increased water for both 2 h and 24 h measurements for three classes of wheat. On the other hand, white salted noodles were brighter ($>L^*$) for both a hard and soft wheat with increasing water absorption (Park and Baik, 2002). Hatcher et al. (1999) also found that recovery percent, maximum cutting strength, and resistance to compression all declined with increased water, but surface firmness did not show a consistent trend with increased water, and Park and Baik (2002) noted that noodles were softer with increased water absorption. The optimum water absorption for noodles across a wide array of hard and soft wheats was negatively correlated (-0.52, $P < 0.05$) with starch damage (Park and Baik, 2002). Since these genotypes varied in starch damage from 6.7% for Hi-Line to 2.2% for HGAB18, it is likely that the softest genotypes with least starch damage would have lower optimum water absorption than Hi-Line with greater starch damage. If water absorption had been optimized it is possible that

Table 6
Means for starch pasting characteristics determined by Rapid Visco Analyzer (RVA) and swelling properties for Hi-Line hard red spring wheat and transgenic isolines over expressing puroindoline a (HGA3), puroindoline b (HGB12), or both puroindoline a and b (HGAB18) averaged over two replications for rain-fed and irrigated environments at Bozeman, MT

Mean	Pasting characteristics				Peak time (min)	Pasting temp. (°C)	Flour swelling volume (ml/g)	Starch swelling volume (ml/g)	Starch damage[a] (%)
	Peak viscosity (RVA units)	Breakdown (RVA units)	Final viscosity (RVA units)	Setback (RVA units)					
Genotype									
H-Line	245	136	244	135	9.57	86.1	16.8	18.3	6.70
HGA3	262	153	253	143	9.62	86.1	19.6	17.4	5.01
HGB12	249	134	251	138	9.72	85.9	20.8	18.2	2.35
HGAB18	255	144	252	140	9.57	85.9	21.4	18.1	2.20
Genotype *P* Value	0.17	0.01	0.77	0.66	0.02	0.50	<0.01	0.15	<0.01
LSD(0.05)	17	10	23	15	0.09	0.4	1.3	0.9	0.64
Environment									
Rain-fed	249	138	247	137	9.65	85.9	19.3	17.7	3.96
Irrigated	256	145	252	141	9.60	86.1	19.9	18.3	4.17
Environment *P* value	0.32	0.35	0.33	0.26	0.18	0.47	0.13	0.08	0.56
$G \times E$ *P* value	0.37	0.45	0.61	0.75	0.98	0.21	0.19	0.11	0.46
CV%	3.9	4.0	5.3	6.4	0.5	0.3	3.8	2.8	14.4

[a] From Martin et al. (2007).

the softest genotypes HGAB18 and HGB12 might have been softer in texture than our results indicate.

A possible explanation for the noodle texture differences at 5 min may be related to flour particle size and starch damage and subsequent water absorption differences among the four genotypes. Since a constant amount of water was added to flour, flours with larger particle size and the greatest starch damage (the hardest grain) absorbed more water during dough formation than flours with smaller particle size and least starch damage (the softest grain) leaving more available water in the noodle dough for flours with smaller particle size and less starch damage. This could lead to more gluten formation during mixing giving rise to increased adhesiveness and hardness for noodles from flours from successively softer grain. Hatcher et al. (2002) evaluated the effect of starch damage and flour particle size on white salted noodle properties by creating three levels of starch damage for small, medium and large particle size. Noodle textural properties improved with decreasing particle size, but showed no consistent trend for starch damage level. Their results pointed to a complex interaction between starch damage and particle size on noodle texture. Noodles were most firm (maximum cutting stress) with high starch damage and small particle size and least firm with high starch damage and large particle size. The grain texture difference brought about by mutations in one of the *Pin* genes has also been associated with differences in occurrence of bound polar lipids on starch (Greenblatt et al., 1995). The four genotypes varying in grain texture in our study could be expected to vary in bound polar lipids as well. It is conceivable that bound polar lipid differences may have a role in noodle hardness differences.

These same genotypes were used to examine the effect of puroindoline over expression on dough and bread quality (Martin et al., 2007). The three transgenic isolines had lower mixograph water absorption than Hi-Line. HGA18 and HGB12 had similar loaf volume but lower than HGA3 which in turn was lower than Hi-Line. It is still unclear whether the reduced loaf volume in transgenic isolines resulted from action of puroindolines per se or indirect effects of starch damage and water hydration. Feiz et al. (2008) used the same flour source as was used in our study to examine differences in starch extractability associated with puroindoline over expression. They found no difference in starch content among the four genotypes, and higher starch extractability from the three transgenic isolines compared to Hi-Line.

Over expression of *Pina-D1a*, *Pinb-D1a* or both in a hard (*Pina-D1a*/*Pinb-D1b*) wheat gave transgenic isolines with intermediate (*Pina-D1a*,), soft (*Pinb-D1a*) and very soft (*Pina-D1a and Pinb-D1a*) grain texture. The transgenic isolines used provide the advantage of examining differences in grain texture brought about by over expression of puroindolines while other genetic factors are held constant. White salted noodles processed from the four flours showed time-dependent change in brightness (L^*) increased for the flours from softer grain. Noodles processed from softer grain also were more adhesive and firmer than those made from harder grain when cooked for 5 min. But when cooked for optimum time, these noodle texture differences showed the same trend, but were compressed in magnitude. Under these conditions over expression of puroindolines did not enhance white salted noodle quality. In practice hard and soft wheats do differ for protein quantity and quality. Protein quantity and quality may mitigate differences in flour physical characteristics to produce desired textural characteristics of white salted noodles from hard and soft classes of wheat.

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